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Humoral (immunological) responses in female albino rats during rotating magnetic field exposures

K. Reid, H. Falter, and M.A. Persinger

Biochemistry and Behavioral Neuroscience Programs, Departments of Chemistry and Psychology Laurentian University, Ontario P3E 2C6, Canada

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Abstract. Experiments were designed to evaluate the primary and secondary humoral responses to a rotating magnetic field configuration, which is known to evoke significant biobehavioral changes. Ten days after inoculation with human serum albumin and 10 days before a booster, female rats were exposed to either a 0.5 Hz rotating magnetic field (RMF) or to room conditions (control). The lighting schedule was either continuous or involved a light-dark cycle (LD) of 12:12 h. A third group of rats served as colony room controls. Group differences of low statistical significance were found when females were exposed to continuous lighting rather than the LD 12:12 light-dark cycle. However, the effects were considered trivial and not sufficient to explain the previously reported biobehavioral changes evoked by this field configuration.

Key words: Immunology – Human serum albumin (HSA) – Magnetic fields – Rats – Females

Introduction

Pre- and perinatal exposure to 0.5 Hz, rotating magnetic fields (RMF) have resulted in several consistent behavioral and physiological changes (Lafreniere and Persinger 1979; Persinger 1969, 1974; Persinger et al. 1978). The primary changes were enhanced emotionality, reduced thymus but increased testicular weights and alterations in serum albumin/globulin ratios. Many of these effects have been replicated by other similar treatments (McGivern et al. 1990).

One general effect of 0.5 Hz RMF exposures is an inverse relationship between the consequences of adult and pre- or perinatal exposure. For example, prenatally exposed rats display indications of hypothyroidism while adult exposed rats display indications of hyperthyroidism (Persinger 1974). One explanation for this effect could involve RMF-stimulation of immunological processes which affect the developing fetus. To test this hypothesis, we decided to measure the reactivity of the primary and secondary humoral response in female adult rats. Considering the size of the typical RMF effect, any significant contribution from the humoral immune system should be readily apparent.

Offprint requests to: M.A. Persinger, Neuroscience Laboratory, Department of Psychology, Laurentian University, Sudbury, Ontario P3E 2C6, Canada

Materials and methods

Animals. A total of 42 female albino Wistar rats, obtained from Charles River Suppliers (Quebec), served as subjects. They were 90–100 days of age at the beginning of the study.

Procedure. After about 2 weeks of habituating to the colony condition each rat was injected subcutaneously (s.c.) in the right flank with 0.1 ml of a 10 μg/ml solution of human serum albumin (HSA) in normal saline plus 0.1 ml of complete Freund's adjuvant. In experiments I and II, the rats were assigned to one of three groups (6–8 rats/group but housed as 2–3 rats/cage). One group was placed within the same 0.5 Hz rotating magnetic field (RMF) that has been used previously. Within the exposure the field strengths ranged from 10 to 30 gauss (Persinger 1974). A second group (room controls) was housed 5 m away within the same room; at this distance the 0.5 Hz magnetic field source was masked within the ambient static field. Other experiments (Persinger 1974) had shown that rats exposed in this region did not differ in responsiveness from rats that had been maintained in the apparatus when the magnets were removed but the motor was operational. Both groups were maintained in plastic cages which were similar to those used previously (Persinger et al. 1978). The third group remained within their home cages within colony housing (colony controls). Food and water were available ad libitum. The light-dark regime for the colony was 12:12 h (onset 0800 h local time). In experiment I a 12:12 h light regime was also used while in experiment II both the RMF-exposed and control groups were exposed to continuous light. All rooms were maintained by thermostat between 21° and 23° C.

Ten days later (20 days since the inoculation) each animal was placed in a restraint cage and tail blood was quickly taken from the tail vein by micropipettes. This was called the baseline measure for antigen-binding capacity (ABC). An identical amount of HSA as described previously (booster) was then injected. Consequently blood samples were taken 5, 10 and 15 days after the booster.

Determination of antigen-binding capacity of rat sera. Blood samples were taken from the tail vein and allowed to clot for 15 min. Serum was separated from the cellular components by centrifugation at $3000 \times g$. The serum was stored at 4° C until used, usually within a few days. HSA was iodinated with ^{125}I by the lactoperoxidase method according to the procedure provided by the manufacturers of the radioiodination reagent (Bio-Rad Laboratories, Richmand, California). The iodinated HSA was separated from unreacted iodine by chromatography over a column of G-25 Sephadex. The iodinated HSA was diluted with non-radioactive HSA to give a final concentration of 1.1 μg/ml.

The antigen-binding capacity of sera was determined by a modification of a method described by Farr (1958). Test serum or appropriate dilutions of test serum (20 μl), carrier serum from control animals (20 μl), ^{125}I-HSA, 1.1 μg/ml (20 μl) and phosphate buffer, pH 7.2, 0.15 mol/l (60 μl) were incubated for 2 h at 37° C. Phosphate buffered saline, pH 7.2 (380 μl) was added followed by 500 μl of saturated ammonium sulfate, mixed and allowed to stand for 15 min at room temperature. The solution was centrifuged for 30 min at room temperature, the supernatant discarded and the precipitate washed with 50% ammonium sulfate (500 μl). The amount of radioactivity contained in the precipitate was then counted with a gamma counter.

The percent of precipitate, corrected for non-specific binding, for each series of dilutions was plotted on a logarithmic scale against the inverse of the dilution. The dilution required to produce 33% precipitation, in the region of antigen excess, was determined by interpolation. The antigen-binding capacity, expressed in micrograms of HSA per ml of serum was calculated according to the formula:

ABC = 0.33 (μg ^{125}I-HSA) (50) (l/dilution)

For those serum samples where antibody levels were not high enough to yield 33% precipitate with neat serum but which yielded more than 10% precipitation, antigen-binding capacity was calculated by the formula:

ABC = (μg ^{125}I-HSA) (% precipitation/100) (50)

Sera of animals that gave less than 10% precipitation were identified as non-responders and not used in further studies. Our a priori definition of a responder to HSA was a level of at least 0.11 μg/ml of antigen-binding capacity (ABC) on the day of the baseline sample.

Results

The means and standard deviations for the log (base 10) of the ABC for the baseline and for days 5, 10 and 15 after the booster for the three groups are shown in Table 1. MANOVA for experiment I indicated no statistically significant group differences [$F(2,15) < 1$, $P > 0.05$]. The differences between days for the ABC values was significant [$F(3,40) = 126.80$, $P < 0.001$]; however the treatment by day interaction was not significant [$F(6,40) < 1.0$]. The group differences were not statistically significant in experiment II [$F(2,15) = 1.30$, $P > 0.05$] while the increase in ABC in response to the booster was again observed [$F(3,45) = 159.1$, $P < 0.001$]. The field condition by days interaction was significant [$F(6,45) = 2.71$, $P = 0.03$]. This was due primarily to the alterations in ABC in the sham field-exposed group compared to the RMF-exposed and colony control groups.

Because of the discrepancy in the binding capacity between the two experiments, as indicated by the colony controls, each rat's ABC was converted to a standardized score. Consequently, each rat's ABC was expressed as a T-score (mean = 50, SD = 10) with respect to other rats for that sample (day) and experiment. MANOVA as a function of the three conditions, two experiments and four repeated measures revealed no statistically significant main effects (all Fs < 1). The only statistically significant ($P = 0.03$) interaction was between experiments, field conditions and repeated measures (days). This effect [$F(6,78) = 2.48$] was due to the relative immunofacilitation of the RMF-exposed groups (T-score = 57) within the continuous light environment for the peak levels of the primary response relative to the room controls (T-score = 44); for comparison the value for the colony room controls (12:12 LD) was 50. The eta coefficient, which is routinely calculated by SPSSX for correlational equivalents when some variables are nominal rather than interval, indicated that the treatment differences explained 12% of the variance in ABC values.

Table 1. Means and standard deviations for the antigen-binding capacity (log base 10) for female rats exposed to the various magnetic field and experimental conditions

Time of serum sampling	Colony controls		Sham field		RMF	
	Mean	SD	Mean	SD	Mean	SD
Experiment I						
Baseline	0.6	0.5	0.4	0.4	0.5	0.4
Day +5	2.0	0.5	2.0	0.2	2.1	0.7
Day +10	2.3	0.4	2.2	0.2	2.5	0.2
Day +15	2.5	0.4	2.3	0.3	2.5	0.3
Experiment II						
Baseline	0.5	0.5	0.2	0.6	0.8	0.5
Day +5	1.8	0.4	1.6	0.3	1.7	0.2
Day +10	1.9	0.3	1.8	0.4	1.8	0.1
Day +15	2.0	0.3	1.7	0.3	2.0	0.4

Discussion

These results are commensurate with the patterns reported in our previous studies. Statistically significant changes associated with RMF exposure are optimally observed when RMF is coincident with *continuous* light conditions. In addition, the major consequence of the RMF exposure appears to be the resynchronization of the consequences of removal from the normal colony and LD 12:12 h environment. Comparable patterns were noted with the levels of circulating thyroxine (Lafreniere and Persinger 1979).

The relatively small magnitude of the RMF effect upon the female's secondary humoral response is not sufficient to explain the powerful effects of prenatal exposure upon adult emotionality (Persinger 1969), serum lactic acid dehydrogenase activity (Persinger et al. 1978)

and the weight of accessory sex organs (Persinger 1974). Direct effects of the rotating magnetic fields upon the developing fetus are more likely explanations.

The relative immunofacilitation of the primary humoral response but not the secondary humoral response of the female rats exposed to the RMF occurred during the continuous light condition. This suggests that coincident ELF (extremely low frequency) magnetic field exposures might affect the initial antigen reaction rather than the processes associated with hypersensitivity. This weak effect, in conjunction with alterations in activity and metabolic activity (Persinger 1974), is an expected general correlate of discrepancies in circadian cycles. Because the primary response contains substantially different ratios of globulins (i.e. more IgM) than the secondary (more IgG) response, the possibility of differential effects upon classes of globulins cannot be eliminated at this time.

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